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Stress Indicators, Immunity and Performance of Quail during Growth Period with the Supplementation of Moringa Leaf Meal (*Moringa oleifera* L.) in Feed

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ABSTRACT

Moringa (*Moringa oleifera* L.) is a plant that contains active compounds, so it has the potential as a source of natural antioxidants to improve animal health. This research aims to analyze the supplementation of Moringa leaf meal on stress indicators, immunity and performance of quail in the growth period. This research used 120 quails with a completely randomized design (CRD) consisting of 4 levels of treatment with the supplementation of moringa leaf meal to the feed and 3 replications. The treatment levels included P0 (without moringa leaf meal), P1 (feed + 2.5% moringa leaf meal), P2 (feed + 5% moringa leaf meal) and P3 (feed + 7.5% moringa leaf meal). The data obtained were analyzed using ANOVA (Analysis of variance) and descriptive analysis. The results showed that the supplementation of moringa meal to quail feed can reduce stress, improve immunity, and increase quail body weight gain. In conclusion, the supplementation of moringa meal reduces stress, increases immunity and body weight gain with the optimal level achieved at the 5% level of supplementation (P2).

Key words: Growth, Immunity, Moringa, Quail, Stress.

INTRODUCTION

Quail (*Coturnix coturnix Japonica*) is one of the poultry with a fast production period and easy to cultivate. Quail egg production reaches 250-300 eggs/head/year (Mardewi et al. 2021). Quail produces a potential food source of animal protein. Quail, like other poultry, has a higher metabolic rate than other livestock. In supplementation to this, poultry have a high body temperature, the body surface is covered with feathers and does not have sweat glands. This condition makes quail vulnerable to heat stress (Qaid and Al-Garadi 2021).

The optimal temperature for raising poultry is 20-24°C (Ulupi et al. 2016). The ambient temperature in Indonesia ranges from 26-33°C, especially during the day when it can reach 35°C (BMKG 2023). This temperature is far above the comfort zone of poultry. This condition is exacerbated by the average air humidity in Indonesia reaching 81% (BMKG 2023). According to Santos et al. (2019), the optimal humidity for quail rearing ranges from 50-70%. Temperatures and humidity that exceed the comfort zone of poultry cause heat stress. Poultry suffering from heat stress will experience panting (Habeeb et al. 2018; Al-Suwailem et al. 2024). Panting is a condition of an accelerated respiratory system. This mechanism is taken to maximize heat expenditure, but on the other hand the body's oxygen adequacy is not met properly. This is because the inhaled oxygen has not had time to be utilized and then released again to maximize heat expenditure. This condition causes oxidative stress.

Oxidative stress is a condition when the amount of free radicals exceeds the antioxidant capacity in the body (Pizzino et al. 2017). Free radicals are by-products of oxygen metabolism. During heat stress, there is an increase in oxygen consumption and metabolism, resulting in increased free radical production (Slimen et al. 2014). This condition is not matched by an increase in antioxidant production in the body, triggering oxidative stress. Oxidative stress can affect the body's resilience. During oxidative stress, glucocorticoid hormones increase, which can inhibit lymphocyte synthesis. Thus, the concentration of lymphocytes in the circulating blood will be low. Low concentrations will decrease lymphocyte activity and impair immunity. According to Oluwagbenga and Fraley (2023) decreased immunity is due to decreased production of antibodies and cytokines by lymphocytes. As a result,

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there is a decrease in immunity so that livestock are more susceptible to infection. Overall, this condition will certainly reduce performance (Oke et al. 2024). One of the efforts to overcome oxidative stress is by providing antioxidants.

Moringa is one of the plants that has potential as a source of antioxidants because it contains active compounds. These compounds can protect body cells from oxidative damage (Srivastava et al. 2023). Pop et al. (2022) stated that Moringa contains bioactive compounds such as flavonoids, phenols, saponins and tannins. Moringa also contains high iron (Fe), reaching 10.3mg/100g (Oluduro 2012). Fe plays a role in binding oxygen to hemoglobin. Given the high metabolism of quail, it requires more oxygen. Feeding Moringa leaf meal increases the Fe content of the feed. Increased Fe consumption increases the synthesis and ability of hemoglobin to bind oxygen (Nurhayati et al. 2023).

Nowadays, Moringa has been used as a supplement in feed to address oxidative stress. Nkukwana et al. (2014) examined the feeding of moringa leaf meal in broiler diets on performance. The levels given were 0, 1, 3 and 5%. The best feed efficiency value was achieved at the 5% level, namely 1.43. Bidura et al. (2020) examined the supplementation of moringa leaf meal in the feed of 30-week-old laying hens on production performance. The levels given were 2, 4 and 6%. The best feed conversion was achieved at the 6% level, namely 2.01. Information on the supplementation of moringa meal on stress indicator variables, immunity and performance of quail in the growth period is still difficult to find. Therefore, the purpose of this research was to analyze the supplementation of moringa leaf meal in feed on stress indicators, immunity and performance of quails in the growth period.

MATERIALS AND METHODS

Ethical approval

This research has received ethical approval and animal welfare license from the Animal Ethics Committee School of Veterinary Medicine and Biomedical Science IPB University (Access No. 197/KEH/SKE/IV/2024).

Time and location of research

This research was conducted from July to August 2024. The meal was analyzed for phytochemical content at the BBPSI Postharvest Agriculture Laboratory, Bogor, Indonesia. Quail rearing was carried out at Arkan Quail Farm, Ciampea District, Bogor Regency, Indonesia. Quail blood and liver samples were taken at the end of rearing when the quail were 5 weeks old. Blood hematology testing was analyzed at the SKHB IPB Research and Diagnostic Laboratory. SOD and MDA assays on liver were analyzed at the Biochemistry Laboratory. Immunity testing was analyzed at the Medical Microbiology Laboratory, SKHB IPB.

Experimental design

The research used a completely randomized design (CRD). The treatment was the supplementation of moringa meal, which consisted of 4 levels. Each treatment was repeated 3 times. The level of moringa meal was modified from the research of Nkukwana et al. (2014). The treatment arrangement of moringa meal supplementation in feed is as follows:

P0: Commercial feed without the supplementation of moringa meal (control);
P1: Commercial feed with 2.5% moringa meal,
P2: Commercial feed with 5% moringa meal; and
P3: Commercial feed with 7.5% moringa meal.

The tools used included cages and equipment (feeders, water gallons, incandescent lamps, hygrometers and tools for laboratory analysis). This research used 120 quails aged 2 weeks, moringa leaf meal, water, feed and materials for laboratory analysis. The first two weeks of rearing used starter period broiler feed with 20% protein content (SNI 8173-2:2022). The last one week used New Hope P100 feed with 20% protein content and metabolic energy of 2800Kcal/kg.

Data observed included stress indicators including oxygen saturation, H/L ratio, liver SOD and MDA levels. Immunity included white blood cell count and differentiation and *Salmonella pullorum* bacterial mortality rate. Quail performance included feed consumption, body weight gain, feed conversion and mortality.

Procedures

Preparation of moringa leaf meal

The preparation of moringa leaf meal includes sorting, cleaning and frying (40-45°C for 16h). Moringa leaves were ground and sieved with a particle size of 300 mesh.

Phytochemical analysis of moringa leaf meal

Moringa leaf meal was analyzed for phytochemical contents including flavonoids, phenols, tannins, and saponins using Stankovic's method (2011).

Maintenance, performance recording and blood and liver sampling

The cages used were $100\times75\times180cm^3$ in size. The number of cages used was two cages. Each cage consisted of 6 plots, making a total of 12 plots. Each cage plot was filled with 10 quails. Before use, the cages were cleaned and disinfected. Quail were reared from the beginning of week 3 to the end of week 5. The average initial body weight of quail was 64.5 g/head. Quail were randomly assigned to cages. Feed and drinking water were given *ad libitum*. Temperature recording was carried out daily in the morning (06.00-07.00am), afternoon (12.00-13.00pm) and evening (16.00-17.00pm).

Body weight gain was obtained from the difference between final weight and initial weight. Feed consumption was obtained from the difference between the amount of feed given and the remaining feed each day. Feed conversion value was obtained by calculating the total feed consumption during the research divided by the total body weight gain. At the end of week 5, 12 blood and liver samples were taken.

Stress indicators

Stress indicators observed included oxygen saturation, H/L ratio and quail liver SOD and MDA levels. Oxygen saturation was observed at 35 days of age using pulse oximetry. The H/L ratio was obtained from the observation of leukocyte differential. Superoxide Dismutase (SOD) was analyzed referring to the method (Maskar et al. 2015). Malondialdehyde (MDA) was analyzed according to the method of Ulhusna et al. (2019).

Immunity

Immunity was observed through white blood cell observation and challenge test using *Salmonella pullorum* bacteria. White blood cell observations were made by counting the number of leukocytes and their differentiation. The number of leukocytes was counted using a Neubauer counting chamber. Leukocyte differentiation was observed using blood review preparations. The challenge test was performed according to the method of Jackson et al. (1998) where blood was challenged with *Salmonella pullorum* bacteria (10^8cfu/mL) and then calculated the percentage of bacterial death. The percentage of bacterial death was calculated based on the initial cfu count minus the final cfu count, divided by the initial cfu count and multiplied by 100.

Data Analysis

Data from the research were analyzed using Analysis of variance (ANOVA) with the help of SPSS software version 25. If there was a significant effect between treatments, it was further tested using the Duncan test (Gaspersz 2012). Data on stress indicators, immunity and feed conversion were analyzed descriptively.

RESULTS

Environmental temperature

Based on observations, the temperature of the quail rearing environment was 25.3-28.5°C in the morning, 31.0-39.3°C in the afternoon and 28.1-33.3°C in the evening. These temperatures were above the comfort zone of the quail livestock, resulting in oxidative stress.

Phytochemical content of moringa leaf meal

The phytochemical content of moringa meal includes flavonoids, phenols, saponins, and tannins. (Table 1) shows the observation of the phytochemical content of moringa meal. Phytochemical analysis of moringa leaf meal shows that moringa leaf meal contains 0.87% flavonoids, 2.32% phenols, 1.63% saponins and 8.52% tannins.

Table 1: Phytochemical content of moringa leaf meal

Phytochemical Content	Results (%)
Flavonoids	0.87
Phenol	2.32
Saponin	1.63
Tannin	8.52

Stress indicators

Quail stress indicators were identified based on oxygen saturation, H/L ratio, liver SOD, and MDA levels. The results of this test can be seen in Table 2. The observation of stress indicators showed that oxygen saturation in group P2 (90.53%) was higher than group P0 (82.60%). The P2 group (0.51) obtained the lowest H/L ratio. The highest SOD levels were obtained in group P2 (57.08units/mL). MDA levels in group P2 (0.09nmoL/mg) were lower than those in group P0 (0.13nmoL/mg).

Table 2: Stress indicators of quail supplementation moringa leaf meal in the feed

Variable	P0	P1	P2	P3
Oxygen saturation (%)	82.60±1.96	85.93±2.50	90.53±1.74	88.06±1.10
H/L	0.56±0.03	0.53±0.01	0.51±0.01	0.52±0.02
Liver SOD (unit/mL)	51.89±5.40	55.01±5.72	57.08±7.88	56.39±4.20
Liver MDA (nmoL/mg)	0.13±0.01	0.11±0.01	0.09±0.01	0.10±0.01

P0: control; P1: moringa leaf meal 2.5%; P2: moringa leaf meal 5%; P3: moringa leaf meal 7.5%.

Immunity

Immunity is observed through white blood cell count, leukocyte differentiation and clearance test using *Salmonella pullorum* bacteria. Leukocyte differentiation includes lymphocytes, heterophils, monocytes, eosinophils, and basophils. Table 3 displays the results of these observations. The mean white blood cell counts ranged from 19.98.98-21.16× $10^{3/mm3}$; lymphocytes 58.00-59.56%; heterophils 30.50-32.60%; monocytes 3.33-4.56%; eosinophils 4.56-5.20 and basophils were not detected. The death rate of *Salmonella pullorum* bacteria was highest in group P3 (99.96%).

Quail performance

Quail performance observed included feed consumption, body weight gain, feed conversion and mortality. The observation results are presented in Table 4. Statistically, feed consumption (19.38-20.10g/head/day) was not significantly different (P>0.05). Body weight gain (68.60-76.53g/head) was statistically significantly different (P<0.05). The highest feed conversion value was obtained in group P0 and the lowest in group P2.

DISCUSSION

Phytochemical content of moringa leaf meal

Flavonoids, phenols, saponins, and tannins were found in Moringa leaf meal. Phenols are secondary metabolite compounds of plants that can act as antioxidants (Rahman et al. 2022). Flavonoids are phenolic compounds, so they have antioxidant properties. When flavonoids are oxidized by radicals, they produce more stable and less reactive compounds that slow down the rate of autooxidation (Hassanpour and Doroudi 2023). Tannins are compounds that have hydroxyl groups, easily oxidized so they have high antioxidant activity (Tong et al. 2022; Bebas et al. 2023). Saponins are not only known for their antioxidant activity, but for also their role as antibacterial. Saponins cause damage to the permeability of bacterial cell walls until the death of bacteria (Anggraini et al. 2019). It is expected that moringa leaf meal has the potential to overcome oxidative stress.

Stress indicators

Oxygen saturation is a method to measure the percentage of oxygen in the blood (Chakravarty et al. 2022). The supplementation of moringa leaf meal in feed increased oxygen saturation compared to the control. According to Abu et al. (2024), oxygen saturation below 90% is an indicator of hypoxemia, which is low levels of oxygen in the blood. This shows that only the supplementation of 5%

Table 3: Immunity of quail supplementation moringa leaf meal in feed

Variable	P0	P1	P2	P3
Leukocytes (10^3/mm^3)	21.16±0.22	20.41±0.71	20.86±2.17	19.98±0.64
Leukocyte differentiation (%)				
Lymphocytes	58.00±2.64	59.00±1.00	59.56±1.40	59.00±0.90
Heterophiles	32.60±1.15	31.66±1.15	30.50±0.78	30.63±0.80
Monocytes	4.16±1.76	3.33±0.57	4.33±0.57	4.56±0.37
Eosinophils	4.56±0.40	5.03±0.76	5.20±0.91	4.99±0.72
Basophils	ND	ND	ND	ND
Salmonella pullorum mortality (%)	78.30±10.48	88.18±9.88	99.64±0.01	99.96±0.02

P0: control; P1: moringa leaf meal 2.5%; P2: moringa leaf meal 5%; P3: moringa leaf meal 7.5%; ND: not detected.

Table 4: Mean performance of quail supplementation moringa leaf meal in the diet

Variables	P0	P1	P2	P3
Feed consumption (g/head/day)	20.10±0.58	19.84±0.79	19.57±0.33	19.38±1.12
Body weight gain (g/head)	68.60±4.55a	72.76±1.76ab	76.53±0.81b	73.83±2.66ab
Feed conversion	6.15±0.58	5.72±0.20	5.37±0.14	5.51±0.46
Mortality	0	0	0	0

Different alphabets in the same line indicate significant differences (P<0.05); P0: control; P1: moringa leaf meal 2.5%; P2: moringa leaf meal 5%; P3: moringa leaf meal 7.5%.

moringa meal (P2) is able to maintain oxygen saturation within the normal range. Normal oxygen saturation indicates that oxygen sufficiency in the body is well achieved. The supplementation of moringa meal increases the Fe content of the feed. Fe plays a role in binding oxygen to hemoglobin (Ahmed et al. 2020) so that it can maintain oxygen saturation in a normal state despite high ambient temperatures.

The H/L ratio is a hematological index that can be used as an indicator of stress (Skwarska et al. 2022). When stressed, the endocrine system secretes stress hormones, one of which is glucocorticoid. This hormone causes an increase in heterophils and a decrease in blood lymphocytes as a defense mechanism which ultimately increases H/L (Wasti et al. 2020). The normal value of H/L according to Thrall et al. (2012) is 0.45-0.5. The H/L ratio in this research ranged from 0.51-0.56. This value indicates that the quail in this research experienced stress. The supplementation of moringa leaf meal was able to reduce the H/L ratio. The supplementation of 5% (P2) moringa meal in the feed resulted in the lowest H/L ratio. The lower the H/L, the lower the stress level. This is due to the content of bioactive compounds in moringa. The supplementation of moringa level increases the bioactive components contained in the feed. Bioactive compounds can increase heat resistance, antioxidant status, and immune function (Guo et al. 2023).

SOD is an endogenous antioxidant that catalyzes the dismutation of free radicals through oxidation-reduction reactions (Younus 2018). The supplementation of 5% moringa meal in the feed obtained the highest liver SOD levels. This condition indicates high levels of endogenous antioxidants that can increase the quail's ability to fight oxidative stress. Flavonoids can indirectly neutralize the toxic effects of free radicals. Flavonoids can increase antioxidant gene expression through the activation of nuclear factor erythroid 2 related factor 2 (Nrf2) resulting in an increase in SOD (Butarbutar et al. 2016). Flavonoids are also able to donate hydrogen atoms to free radicals. This will reduce free radical chain reactions and produce compounds that are more stable and less susceptible to autooxidation (Kumar and Pandey 2013).

MDA is one of the biomarkers of oxidative stress resulting from lipid peroxidation (Cordiano et al. 2023). The results showed that the supplementation of moringa meal in feed can reduce MDA in quail liver. The lowest MDA value in this research was obtained at the level of 5% moringa meal (P2). Low MDA levels are a sign that the level of oxidative stress is low. Lower MDA is also related to increased SOD levels. SOD is able to catalyze the change of superoxide radicals into more stable molecules. This effectively reduces lipid peroxidation caused by free radicals, thus lowering MDA levels (Tariq et al. 2022).

The P2 treatment produced the best physiological condition of the four stress indicators observed. Although the highest level of moringa meal was given in P3, the best results were obtained in P2. This is due to the higher tannin content in P3. The higher the supplementation of moringa meal means the greater the concentration of tannins. Tannins can bind to proteins and minerals, forming complex compounds that are difficult to absorb by the body (Naumann et al. 2017).

Immunity

White blood cells are an important component of the immune system that works through phagocytosis and antibody formation (Marshall et al. 2018). The observation of leukocyte count in this research was in the normal range. According to Mahmoud et al. (2013), the average of normal leukocytes in quail is in the range of 17,20-22,91×10^3m^{-3}. This indicates that leukocytes have the same good potential in maintaining immunity. However, the supplementation of moringa meal in feed has the potential to increase immunity. The presence of metabolic compounds that function as antioxidants and antibacterials has the potential to strengthen the immune system of quail (Mahfuz and Piao 2019).

Leukocyte differentiation includes lymphocytes, heterophils, eosinophils, and basophils. Leukocyte differentiation in this research has a percentage that is almost the same as the research of Mahmoud et al. (2013) and Maheshwari et al. (2017). This condition indicates that the quails in this research, both in the control group and with the supplementation of moringa leaf meal in the feed, have the same immunity. This means that the quail in this research have the same good potential in fighting exposure. The presence of basophils in this research was not detected. Although not detected, it does not mean that it does not contain basophils but the concentration is very small. Basophils are needed because they contain heparin which can inhibit the blood clotting process (Okpalugo and Ogwu 2016).

The level of resistance of the quail body was also evaluated with blood samples challenged with *Salmonella pullorum* bacteria. In fact, quail with moringa meal added to the feed had a higher ability to kill bacteria. This ability increased as the level of moringa meal increased. The content of active compounds, especially saponins in moringa plants, functions as an immunomodulator and anti-bacterial (Timilsena et al. 2023). Increasing the level of supplementation of moringa meal will increase the intake of active compounds, especially saponins. Saponins act as anti-bacterial by denaturing proteins that result in membrane damage to bacterial death (Khan et al. 2018).

The supplementation of moringa increases immunity so that the increase in glucocorticoids which are immunosuppressants can be suppressed (Jia and Zhang 2022). Leukocyte concentration and differentiation have the same potential in maintaining immunity. However, the ability to kill bacteria after being tested using *Salmonella pullorum* bacteria, the supplementation of 5% and 7.5% moringa meal in feed showed the best results.

Quail performance

The supplementation of moringa meal in feed had no effect ($P>0.05$) on feed consumption. According to Barzegar et al. (2020), the main factors affecting feed consumption are the energy content of the feed and environmental temperature. The energy content of the feed and the uniform environmental temperature in this research caused the resulting feed consumption to be the same.

The supplementation of moringa meal significantly affects ($P<0.05$) the body weight gain of quail in the growth period. The best results were obtained with the supplementation of 5% (P2) moringa meal in the feed. The supplementation of moringa meal increases the protein content of the feed. Protein is an essential macronutrient in the growth process by meeting the metabolic needs of amino acids for tissue growth (Xiong et al. 2023). The high oxygen saturation at P2 also indicates that oxygen needs for metabolic processes are well met. An optimal metabolic process will reduce the level of stress experienced by quail, especially in high temperature conditions. This condition supports a faster growth rate and results in higher body weight gain.

Feed conversion is an indicator used to show the efficiency of feed utilization (Yi et al. 2018). The supplementation of moringa meal in feed gives positive results with lower feed conversion values. The best feed conversion was obtained with the supplementation of 5% (P2) moringa leaf meal in the feed. This condition can be achieved because P2 obtained the best oxygen saturation and lower stress levels. This will certainly increase the ability of quail to convert the feed consumed for growth. Despite the heat stress condition, no dead quails were found.

Although the supplementation of moringa meal was highest in P3, the best performance was obtained in P2. Along with the increase in moringa feeding level, there was also an increase in tannin content. Tannins can bind, precipitate and inhibit protein synthesis (Sunani and Hendriani 2023). This condition causes the protein that should be metabolized by the body cannot be absorbed optimally for the growth process.

Conclusion

The supplementation of moringa leaf meal in quail feed for the growth period can reduce oxidative stress, increase immunity and body weight gain. The optimal level was achieved at the 5% feeding level (P2).

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Author's Contribution: Research design; NU and AS; Data collection: AS; Data analysis, data validation and data interpretation; NU and HM; Original draft: AS; Critical review and editing: NU and HM.

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